Comparative study of Biocorrective Protein-Peptide Agent to Improve Quality and Safety of Livestock Products

Natural immunostimulators obtained over Sus scrofa tissue extraction with the use of water with modified isotope composition (RSF grant No. 15-16-00008)

Ekaterina R. Vasilevskaya
Study devoted to analyze water with a modified isotope (D/H) composition (WMIC) influence on Sus Scrofa tissues extracts.

Tasks:
• understand WMIC influence on the protein extractability, peptide and protein profiles;
• Comparative study of complex extracts obtained from Sus scrofa immunocompetent organs prepared with distilled water and WMIC in vivo

WMIC – deuterium depleted water with deuterium concentration 40 ppm
DW – standard distilled water with deuterium concentration 140 ppm
RAW MATERIALS CHOICE

BONE MARROW
(hematopoietic stem cell)

Predecessor of the myeloid series

- Monocyte
- Macrophage
- Neutrophil
- Eosinophils
- Basophil
- Mast cell

Predecessor B-lymphocytes
(immune memory)

B-lymphocytes maturation

Predecessor T-lymphocytes
(antigen-specific)

THYMUS

T-cells differentiation
(CD4/CD3/CD8/TcR)

Mature B- and T-lymphocytes circulation
(CD20/CD14/IgM/IgG)

SPLEEN

Cell-mediated immunity (C):
- T-helpers (Th);
- T-killers (Tc);
- T-suppressors (Ts);

Humoral immunity (H):
- Ig, lymph nodes

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Main Sus Scrofa immune organs – spleen, thymus and lymph nodes as potential tissues containing compounds with immunoregulatory properties

V.M. Gorbatov VNIIMP, 2016 - Moscow
EXTRACTION ALGORITHM

Grinding
- Knives with ceramic coating

Extraction
- 4°C, 0,9 % solution NaCl - WMIC, 4 hours
- 4°C, 0,9 % solution NaCl - DW, 4 hours

Centrifugation
- 3500 Rev/min, 8 min, Plastic tubes

Ultrafiltration
- Pressure 2.5 bar
  Polyethersulfone membranes with plastic fittings and tanks

Lyophilic drying
- Pressure 3,3 Pa, T = (-41±1°C)
- Glassware

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Increase protein concentration from 15 to 50 %
1. Standard molecular weight (130 kDa– 10 kDa);
2. thymus extract (WMIC);
3. spleen extract (WMIC);
4. lymph nodes extract (WMIC);
5. thymus extract (DW);
6. spleen extract (DW);
7. lymph nodes extract (DW).

Major bands – 13 kDa, 16 kDa, 27 kDa, 43 kDa, 70 kDa, 98 kDa

Leucocyte antigen
Cell tumor antigen
Interleukins
2D ELECTROPHORESIS (O'Farrell) OF EXTRACTS MIXTURE (WMIC)

Identification of protein fractions was performed on DE after trypsinolysis by MALDI-TOF/MS and MS/MS mass spectrometry on Ultraflex MALDI-TOF mass spectrometer

- Marked differences: proteins involved in the immune response

<table>
<thead>
<tr>
<th>№№</th>
<th>Protein</th>
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<tbody>
<tr>
<td>1</td>
<td>Interferon beta protein</td>
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<tr>
<td>2</td>
<td>Gamma-interferon-inducible-lysosomal thiol reductase</td>
</tr>
<tr>
<td>3</td>
<td>Myeloid differentiation primary response protein</td>
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<tr>
<td>4</td>
<td>Interleukin-12 subunit beta</td>
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<tr>
<td>5</td>
<td>Annexin A1</td>
</tr>
<tr>
<td>6</td>
<td>MHC class I antigen</td>
</tr>
<tr>
<td>7</td>
<td>Chemokine-like receptor 1</td>
</tr>
</tbody>
</table>
IMMUNOLOGICAL REACTIVITY IN VIVO

Study of immune corrective effect was carried out with:

ANIMALS
- Male rats Wistar (SPF)
- N= 40, m = 390 ± 10 г

IDF MODEL
- Intraperitoneal injections of Cyclophosphamide (Sigma)
- Dose: 75 мг/кг
- Three times every 72 hours,
- Model complete: 12 days after first injection

IN VIVO RESEARCH
- Group A (n=10) – intact animals
- Group B (n=10) – control animals (IDF model)
- Group C (n=10) – treatment for 20 days with DW extract, 2,67 ml/kg
- Group D (n=10) – treatment for 20 days with WMIC extract, 2,62 ml/kg

METHODS
- Cytometry analysis :
  LYM, MON, GRA; CD4.
- Immunoassay analysis:
  Complement components C_{1q}, C_3, C_4, C_5
IMMUNOPHENOTYPING

Lymphocytes, monocytes, granulocytes content.
A – intact; B – control; C – DW extract; D – WMIC extract.

Increase GRA:
B group by 87 %,
C group by 43 %
D group by 21 %
IMMUNOPHENOTYPING

CD4 content (T-helper cells).
A – intact; B – control; C – DW extract; D – WMIC extract.

Decrease:
B group by 40 %,
C group by 20 %
D group by 11 %
COMPONENT COMPLEMENT IMMUNOASSAY ANALYSIS

1 – intact; 2 – control; 3 – DW extract; 4 – WMIC extract.

Activated cycle of complementary cascade

Decrease C1q:
B group by 25 %,
C group by 22 %
D group by 9 %

Adaptive immune response activation by stimulating C3 and C4 components synthesis
CONCLUSION

• Deuterium depleted water intake led to the increase protein concentration during extraction;

• WMIC has an influence on extraction proteins with low molecular weight (by 15 kDa) in animal tissue extracts (spleen, thymus, lymph nodes)

• Use WMIC as solubilizing agent led to increase of proteins and peptides count that are directly or indirectly involved in the immune response

• In vivo research showed immune system recovery, adaptive immune response and functional activity of nonspecific immune defense
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“Development of innovative natural adaptogenic stimulants of innate (nonspecific) immunity based on species and tissue-specific biomolecules”

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